Master Thesis

MONOMOLECULAR REACTION NETWORKS: A NEW PROOF OF FLUX TRANSITIVITY

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Abstract

In the case of monomolecular reaction networks, we study the network response to perturbations of a reaction rate j^*. Specifically, we describe which other reaction rates j' respond by nonzero reaction flux, at steady state. Nonzero responses of j' to j^* are called flux-influence of j^* on j'. Mochizuki and Fiedler established transitivity of flux-influence for monomolecular reaction networks. We give a new, independent and conceptually simplified proof of that intriguing fact. Our proof uses standard connectivity concepts from graph theory, and Menger's Theorem. Based on the network structure only, this also leads to a simplified characterization of all flux-influenced sets.

Contents

Introduction

The spark for this work comes from a Seminar, *Chemical Reaction Networks*, held by Bernold Fiedler and Isabelle Schneider at the Free University of Berlin during the Winter semester 2014/2015, and specifically from the papers *Sensitivity of chemical reaction networks: a structural approach* by Fiedler and Mochizuki (cfr: [1],[2]).
The main and most important aspect of the analysis of *Sensitivity of chemical reaction networks: a structural approach* lies indeed in the reaction graph approach, in which the chemical reaction networks are modeled by directed graph (cfr also: [3], [4]) and the whole analysis is function-free.
In particular, their approach allows a graph theoretical description of sensitivity of chemical reaction networks. Here, sensitivity denotes the response of steady states to perturbations of reaction rates. The idea then is to analyse which reactions respond to a rate perturbation of a specific reaction. We emphasize that the analysis does not require numerical input but is based on the graph only.
The main theorem of [2] turned out to have a transitive property, which directly implies that reactions influence others in a sort of pyramid structure.
Nonetheless, the proof of transitivity was more involved than expected.

Our goals, strongly connected with each other, are basically three:

1. investigate connections between the above mentioned papers and standard graph theory literature;

2. obtain a new and simpler proof of the transitivity;

3. address a more topological characterization of the relations of influencing and being influenced in the network.

Regarding point 1, the key-role for this specific theorem is played by Menger's Theorem, a classical graph theorem proved in an algebraic curves setting for the first time in 1927 by Karl Menger.
Regarding point 2, the transitivity is proved in a simpler and conceptually more transparent way.
Regarding point 3, we have characterized the whole set of the reactions influenced by a perturbation of a specific reaction.

The thesis is organized as follows:

In Chapter 1 language and notation are set: especially in graph theory, depending on the specific source of literature, words may have indeed slightly different meaning and these differences may sometimes generate theoretical issues.
In Chapter 2 monomolecular reaction networks and the flux-influence theorem are briefly presented as in the paper by Fiedler and Mochizuki (cfr: [2]).
Chapter 3 deals with all the technical tools which have been used for proving the results. It consists of three sections: the first is mainly technical and it introduces some graph tools useful for the proofs of the theorems. When some of these tools appear in the statement of some theorems, we have repeated briefly the meaning of them. The second section is about one of the main conceptual ingredient of our work: Menger's theorem. A straightforward corollary is derived which will be used in one of the main proofs. The third section of this chapter introduces the main tool we have defined for a better comprehension of this specific topic: the obliged elements.
Chapter 4 is the core of our work for this thesis: the flux-influence theorem presented in Chapter 2 is proved to be equivalent to another formulation, which involves the obliged elements defined in Chapter 3. Here Menger's theorem plays a crucial role. After this reformulation, proving the transitive property of the flux-influence theorem is straightforward.
Chapter 5 contains a theorem characterizing precisely the entire set of reactions influenced by a perturbation on a single reaction. Transitivity of the flux-influence relation is proved also in this environment, by showing inclusivity of the influenced sets.
In Chapter 6 we have presented another point of view, which uses a partially different language than ours. This result was first presented by Hiroshi Matano in a talk at the Equadiff Conference 2015 (cfr [5]). This chapter may be read mostly independently from the others.
In Chapter 7 we have listed some straightforward consequences of the work of this thesis that might be useful for readers who are not necessarily mathematicians.
Chapter 8 contains seven examples where we have applied our results.
In Chapter 9 we have discussed some open problems and summarized the thesis.
The appendix contains a glossary of terms used in the main theorems, for easy reference.

Acknowledgment

First of all, I am very grateful to Bernold Fiedler for his continuous support and confidence. I thank Hiroshi Matano for having generously shared many ideas and different points of view.
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Chapter 1

Preambulum

In this chapter we want to collect some common definitions from graph theory. For this purpose, we have mainly followed the book [6], *Graph Theory*, by Harary and the book [7], *Graph Theory with Applications*, by Bondy and Murty.

Graph: A graph Σ consists of a finite nonempty set $\mathbf{V}$ with a prescribed set $\mathbf{E}$ of unordered pairs of elements of $\mathbf{V}$. The elements of $\mathbf{V}$ are called *vertices* and the elements of $\mathbf{E}$ are called *edges*. Thus an *element* of the graph can be a vertex or an edge. The two vertices associated with each edge are called *endpoints* of the edge. The edge is said to *join* or to connect the two endpoints. For these we use letters x, y, z, w for referring to vertices, letters e, i, j, k for referring to edges, and p, q, r, s for generic elements.

Subgraph: A subgraph Θ of a graph Σ is a graph whose vertex set is a subset of the vertex set of Σ, and whose edge set is a subset of the edge set of Σ. In this case we also say that Σ contains Θ and that Θ is contained in Σ.

Walk: A walk of a graph Σ is an alternating sequence of vertices and edges $[..xjyiz..]$ of Σ, where each edge's endpoints are the preceding and following vertices in the sequence. In this notes we work in a slightly different way than in the established graph literature: we consider as a walk also a sequence which has as starting and ending elements indifferently vertices or edges.

Path: A path is a walk in which no vertices and no edges are repeated.

Cycle: A cycle is a walk in which the starting and the ending element coincide but no other vertices and edges are repeated.

Digraph: A digraph (or directed graph) Γ consists of a finite nonempty set $\mathbf{V}$ with a prescribed set $\mathbf{A}$ of ordered pairs of elements of $\mathbf{V}$. The elements of $\mathbf{V}$ are called *vertices* and the elements of $\mathbf{A}$ are called *arrows* (or *directed edges)*. Thus an *element* of the graph can be a vertex or an arrow. Let j be an arrow, and x and y two vertices by it connected, then $j = (x, y)$ is considered to be directed from x to y; $y = h(j)$ is called the *head* and $x = t(j)$ is called the *tail* of the arrow. Moreover, j is said to be an *outgoing* arrow from x and an *ingoing* arrow of y. Let p and q be elements, if a walk made up of one or more successive arrows leads, following the order, from p to q, then it is called a *diwalk* (or *directed walk*) from p to q. Analogously we define *dipath* (or *directed path*) and *dicycle* (or *directed cycle*) as above. A digraph Γ is said to be *strict* if no two arrows with the same two endpoints have the same orientation and there are no self-loops, namely dicycles L=[xjx] where x is a vertex and j an arrow.
We use sometimes the word *undirected* referring to graphs, walks, paths, etc., to underline

that they are not directed.

Subpath: Let γ be a dipath. Let p, q be elements of γ. Let

$$\tilde{\gamma} = \gamma[p, q]$$

denote the subpath of γ which starts at the element p and, following the same sequence of vertices and arrows as γ, ends with q, including p, q. We will largely use this notation also for referring generically to a dipath from p to q.
Moreover, when the situation renders impossible to use the above notation, we will use also the intuitive notations with parenthesis $\gamma(x, y)$, $\gamma(x, y]$, $\gamma[x, y)$ for referring to dipaths from x to y which do not include x and y; x; y, respectively.

Connectivity: A graph Σ (or a digraph Γ) is said to be *connected* if, for every couple of elements p and q, there exists an undirected path from p to q. Conversely, it is said do be *disconnected* if it is not connected. We underline that the definition relies on undirected paths also for a directed graph Γ. We say that Σ (or Γ) is *2-connected* if there not exist elements whose single removal renders Σ (or Γ) disconnected. When we remove a single vertex, here, it is understood that its edges (or ingoing and outgoing arrows) have to be removed, as well. If the connected graph Σ (or digraph Γ) is not 2-connected, we call the elements whose single removal disconnects Σ (or Γ) *cut elements*.

Chapter 2

The flux-influence relation

In this chapter we recall the main theorem on the flux-influence in the case of monomolecular chemical reactions, by Fiedler and Mochizuki, following [2]. We do not aim for a complete recall and we will not go into details. For thorough mathematical details, see [2]. The reader should particularly pay attention to the specific kind of directed graphs which are the monomolecular reaction networks. For the purposes of this thesis, it is crucial that this theorem is understood from a graph point of view more than from a Ordinary Differential Equations point of view. In particular we will omit all the proofs that may be easily found complete in [2]. Nevertheless, the importance and the meaning of these results rely on the chemical consequences and therefore it is still totally worth briefly mentioning the origin of the issue.

2.1 Monomolecular reaction networks

A *monomolecular reaction network* is a strict directed graph Γ with a vertex set $\mathbf{M} \cup \{0\}$ and an arrow set $\mathbf{A}$. The vertices belonging to $\mathbf{M}$ are called *metabolites*. 0 is the *zero-complex* introduced by Feinberg in [3]. According to Feinberg, we shall think the zero-complex as *'a complex in which the stoichiometric coefficient of every species is zero'* (cfr [3], Remark 4.A). The outgoing arrows from 0 are called *feed reactions*, the ingoing arrows towards 0 are called *exit reactions*. Any dipath that has as ending element the vertex 0 is called an *exit dipath*. We use the letter m for metabolites, namely any vertex which is not 0.

For any nonzero metabolite $m \in \mathbf{M}$, let $e_m \in \mathbb{R}^{|\mathbf{M}|}$ be the m-th unit vector, and define moreover $e_0 = 0 \in \mathbb{R}^{|\mathbf{M}|}$. Here, of course, we have implicitely associated a number $m \in \mathbb{N}$ to every metabolite in the finite set $\mathbf{M}$. The ODE for the dynamics of the vector $x{=}(x_m)_{m\in\mathbf{M}}$ of concentrations x_m of the metabolites $m \in \mathbf{M}$ is

$$\dot{x} = g(x) = f(\mathbf{r}, x) := \sum_{j\in\mathbf{A}} r_j(x_{t(j)})(e_{h(j)} - e_{t(j)}).$$

Here $\mathbf{r} = (r_j)_{j\in\mathbf{A}}$ are the reaction rate functions and it is important to underline that we consider them as smooth given parameters.

Moreover, some assumptions are made:

1. positivity of the reaction rate

$$r_j(\xi) > 0 \text{ for } \xi > 0,$$

2. existence of a positive steady state $x^* > 0$, i.e.,

$$0 = g(x^*) = f(\mathbf{r}, x^*),$$

3. regularity of the network at the steady state x^*

$$\det f_x(\mathbf{r}, x^*) \neq 0.$$

   From [2] we know that this assumption implies, from a network point of view, that from every metabolites m there exists an exit dipath $\gamma = \gamma[m, 0]$ from m to 0.

Therefore, it is better to strongly focus on it, a *monomolecular reaction network* is precisely a strict directed graph with a special vertex 0 such that from every other vertex m there exists a directed path from m to 0.

2.2 Recall of the main theorem

Let us explain firstly what perturbation means, from a mathematical point of view.
For any function $\rho \in C^1$, a C^1-small perturbation of $\mathbf{r} = \mathbf{r}^0$ is

$$\mathbf{r}^\epsilon := \mathbf{r} + \epsilon\rho,$$

where ϵ is small and real.
By assumption 3, we can apply the standard implicit function theorem. By using this we are guaranteed that the perturbation induces a response curve $x^*(\epsilon)$, for small ϵ, such that, at $(\mathbf{r}^\epsilon, x^*(\epsilon))$,

$$f_r \cdot \rho + f_x \cdot \tfrac{d}{d\epsilon} x^* = 0.$$

We consider the special case in which $\rho = (\rho_j)_{j \in \mathbf{E}}$ and $\rho_j = \rho_j(x_{t(j)})$ does not modify the reaction network but perturbs reaction j^* only, so that

$$\rho_j(\xi) = 0 \text{ for } j \neq j^*, \text{ and}$$

$$\rho_{j^*}(x^*_{t(j^*)}) = 1.$$

Without loss of generality, we have normalized the perturbation of rate j^*.
Now we can define properly the infinitesimal concentration response $\delta x_m^{j^*}$ of metabolite m at steady state as

$$\delta x_m^{j^*} := \tfrac{d}{d\epsilon}\big|_{\epsilon=0} x_m^*(\epsilon).$$

Moreover, we say that $\delta x_m^{j^*}$ is *algebraically nonzero* if $\delta x_m^{j^*} \neq 0$ as a rational function of the derivatives $r_{jm} := r_j'(x_m^*)$ with $m = t(j)$.

We define the *infinitesimal change of the flux through reaction arrow* j', in response to a rate perturbation of reaction j^*, as

$$\Phi_{j'j^*} := \delta_{j'j^*} + r_{j't(j')}\delta x^{j^*}_{t(j')},$$

where the Kronecker-delta $\delta_{j'j^*}$ accounts for the explicit flux change induced by the external perturbation at j^* in the specific reaction $j'{=}j^*$ only. The second term indicates the flux change in any other reaction j' which is caused by the concentration response $\delta x^{j^*}_{t(j')}$ of the tail reactant $t(j')$ to the external perturbation at j^*.
We denote partial derivatives of reaction rates at the equilibrium x^* by

$$r_{jm} := \frac{\partial}{\partial x_m} r_j(x^*) = \begin{cases} r_j'(x^*_m) & m = t(j) \\ 0 & \text{otherwise.} \end{cases}$$

We then say that a reaction j^* *flux-influences* a reaction j' ($j^* \rightsquigarrow j'$) if the flux response $\Phi_{j'j^*}$ of reaction j' to a rate perturbation as above of reaction j^* satisfies algebraically

$$\Phi_{j'j^*} \neq 0.$$

We can now finally state the flux-influence theorem:

Theorem 2.2.1 (Fiedler&Mochizuki, 2015)**.** *Let the above assumptions hold, and consider any pair of reaction arrows (j', j^*), not necessarily distinct.*
Then j^* flux-influences j' ($j^* \rightsquigarrow j'$) if, and only if, there exist two dipaths γ^0 and γ' for which the following four conditions all hold true:

1. *both dipaths emanate from m^*, the tail of j^*;*
2. *one of the dipaths contains j^*;*
3. *γ^0 is an exit dipath, i.e., it terminates at vertex 0, and $\gamma'{=}\gamma'[m^*, j']$, i.e. it terminates with arrow j', omitting the head vertex of j';*
4. *except for their shared starting vertex m^*, the two dipaths γ^0 and γ' are disjoint.*

For the proof we refer to the paper by Fiedler and Mochizuki [2].

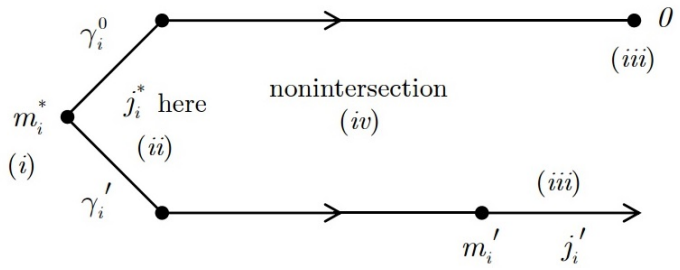


Figure 2.1: *The figure, taken from the reference paper* [2] *by Fiedler and Mochizuki, summarizes the structure of the theorem: from the tail vertex of the influencer reaction j^* emanate two directed dipaths: one leading to vertex 0 and one leading to the influenced reaction j'. One of them contains j^* and they do not intersect apart for the shared starting vertex m^**

We underline the fact that this theorem states, and, importantly, sets a relation which is not numerical but structural. We may think this theorem as a definition of a flux-influence relation in directed graphs of the kind of monomolecular reaction networks, namely strict directed graphs with a special vertex 0, which is reachable by a dipath from any other vertex m.
As we will explain later, the difficulties of proving the transitivity of the flux-influence relation are given by the fact that the theorem deals with two dipaths which, in particular, do not intersect except at the starting vertex m^*. A typical transitivity proof, as done in the main transitivity theorem of the paper [2], has to build two dipaths which do not intersect, from two different pairs of not intersecting dipaths. This is in no way trivial: the issue we will pursue is whether it is necessary to state this theorem by using two dipaths and the answer we did find is that, indeed, it is not.
In the next chapter we define and investigate some tools for dealing better with the directed graphs of the kind of monomolecular reaction networks.

Chapter 3

Technical tools

3.1 Definitions

Our idea for this section is to establish graph theory tools for building bridges between the dipaths formulation of the main Theorem 2.2.1 about flux-influence in Chapter 2 and usual graph theory. We underline again that we generically use letters $i, j, ..$ for arrows, $x, y, ...$ for vertices and $p, q, ...$ for generic elements of the digraph (indistinctly arrows and vertices).

The first issue that arises is the following: let us consider two concatenated dipaths in a graph, i.e., a dipath from x to y and then a dipath from y to z, where x, y, z are vertices. We want to define an operation which allows us to easily obtain an existing dipath from x to z. This will be very useful in our proofs. The first definition, then, is as follows:

Definition 3.1 (Last-minute shortcut and skipped elements)**.** Consider any two dipaths $\gamma_1 = \gamma_1[x, y]$ from x to y and $\gamma_2 = \gamma_2[y, z]$ from y to z, with x, y, z vertices of a digraph. Let w be the last vertex, in the natural order given by γ_2, of the nonempty intersection $\gamma_1 \cap \gamma_2$. Then we call the concatenated dipath

$$\gamma := \gamma_1[x, w)\gamma_2[w, z]$$

the *last-minute shortcut* and we use the notation $\gamma = s(\gamma_1\gamma_2)$.
Moreover, let p be an element (either a vertex or an edge) such that p belongs either to γ_1 or to γ_2 but not to the last-minute shortcut $\gamma = s(\gamma_1\gamma_2)$. We call p a *skipped element*.

Observation 1. The last-minute shortcut is, by construction, a dipath from x to z. Indeed, since w is the last intersection between γ_1 and γ_2 in the order of γ_2, this implies that $\gamma_1 \cap \gamma_2[w, z] = \{w\}$ only, and hence in particular $\gamma_1[x, w] \cap \gamma_2[w, z] = \{w\}$ only. Therefore the sequence $\gamma = \gamma_1[x, w)\gamma_2[w, z]$ counts each elements just once and it is a dipath.

Observation 2. We observe that, in particular, any skipped element lies on a dicycle. Indeed, any skipped element lies, by construction, on a diwalk $\psi = \gamma_1[w, y]\gamma_2[y, w]$ which starts and ends with the same vertex w.

The second issue is to define a subgraph, which is completely reachable from a vertex. Of course this issue is well explored in established graph theory (cfr [6], [7]), but we want to repeat it here and make sure that nothing will be misunderstood.

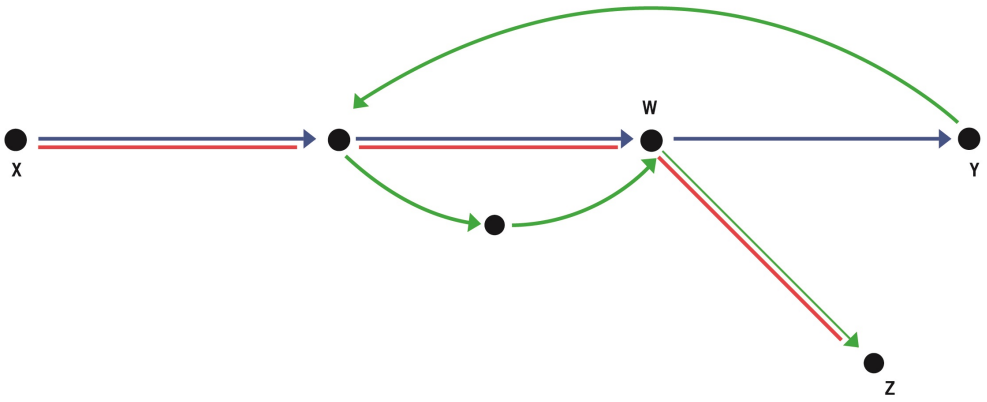


Figure 3.1: *The figure provides an example of the construction of the last-minute shortcut. From a blue dipath from x to y and another green dipath from y to z, we have obtained a third path from x to z, the last-minute shortcut, underlined in red.*

Definition 3.2 (Reachable Subgraph)**.** Let x be a vertex of a directed graph Γ and j an outgoing arrow from x. We define the *reachable subgraph* $R^x(\Gamma)$ *from* x, as the subgraph of Γ which consists in all the elements reachable with a directed path from x.
Moreover, we define the *reachable set* $R^{x,j}(\Gamma)$ *from* x*, starting along* j as the subset of the graph Γ, which consists in all the elements reachable with a directed path from x which has j as first arrow.
We may omit the argument (Γ) when no doubt arises.

We underline again that in a directed path no vertices and edges are repeated.

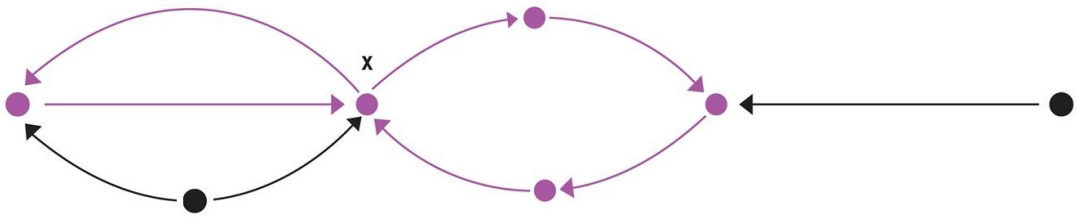


Figure 3.2: *A generic digraph, in purple the subgraph R^x reachable from x.*

Observation 3. With the settings as above, $R^{x,j}(\Gamma)$ is always contained in the $R^x(\Gamma)$. Moreover, it is exactly the same if and only if j is the only outgoing arrow from x.

Observation 4. In the case of monomolecular reaction networks, it is clear that, for any metabolite m, $0 \in R^m$. However it is not true that 0 lies in any $R^{m,j}$, where j is an outgoing arrow of m. The only way to go to 0 from the head of j might be indeed to come back again to m, namely counting m two times, which is not allowed by the definition of a dipath. This idea leads us to the next definition.

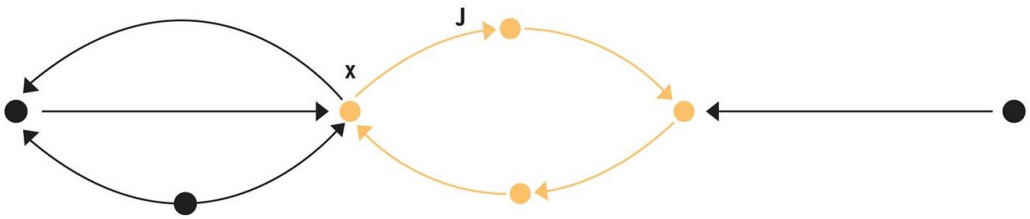


Figure 3.3: *In the same generic digraph as* Figure 3.2*, we have now selected in orange the subgraph $R^{x,j}$ reachable from x with a path starting along j. Note that of course, this turns out to be a subgraph of R^x reachable from x. For any vertex y with n different outgoing arrows i_n, there are n different subgraphs R^{y,i_n}.*

The last definition defines a subgraph of a directed graph given by all the dipaths between two elements of the graph. In particular, it gives a subgraph which has a source and a sink. It is clear, in our application for monomolecular reaction networks, that we will use mainly the vertex 0 as a sink.

Definition 3.3 (xp-direction subgraph)**.** Let x be a vertex of a directed graph Γ and let p be an element reachable from x.
We call *xp-direction* the subgraph made as follow:

$$xp\text{-}direction := \{q \in \Gamma \; s.t. \; \exists \text{ dipath } \gamma = \gamma[x,p] \text{ from x to p which does include q}\}$$

or, analogously, the xp-direction subgraph is given by the union of all the directed paths from x to p:

$$xp\text{-}direction := \bigcup^{\text{ALL}} \gamma[x,p]$$

where $\gamma[x,p]$ is any dipath from x to p.

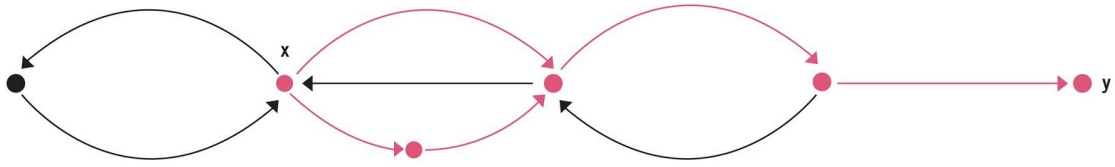


Figure 3.4: *In a generic directed graph, we have selected in red the xy-direction subgraph.*

Observation 5. We note that, by construction, the source x has clearly no ingoing arrows in the xp-direction. Analogously, in the case in which the element p is a vertex, p has no outgoing arrows in the xp-direction.

Observation 6. In a monomolecular reaction network, for j outgoing arrow from m the following statements are clearly equivalent:

1. $j \notin m0$-direction
2. $0 \notin R^{m,j}$

We will use both versions, but mainly the first one, up to Chapter 5.

3.2 Menger's Theorem

This section explains briefly the ideas and developments of a well known theorem by Karl Menger, proved in 1927 and originally stated in the framework of algebraic curve theory (cfr [8]). However, the main applications of this theorem are in graph theory. It was later generalized to the also famous Max-Flow-Min-Cut theorem proved in 1956 by Elias, Feinstein and Shannon, and, independently the same year, by Ford and Fulkerson.
The central idea of this theorem is to set an equivalence between the order of cut-sets (namely, the minimum number of elements whose deletion disconnects the graph) and the number of internally disjoint (directed) paths. Two (directed) paths are called *internally disjoint* if they share starting and ending point only, namely they do intersect just in these points and they do not intersect 'internally'.
There are many versions of Menger's Theorem, both for directed and undirected graphs, both for cut-sets consisting just of vertices and cut-sets consisting just of edges/arrows. We will not pursue here further explanations, which, along with more references, can be completely found in [6] [7], [9] and [10].

We will use the following vertex version of Menger's Theorem for directed graph.

Theorem 3.2.1 (Menger, 1927)**.** *Let x and y be vertices in a digraph Γ, such that y is reachable from x. Then the maximum number of dipaths from x to y internally disjoint is equal to the minimum number of vertices whose deletion renders the xy-direction disconnected.*

And we now state a simple corollary.

Corollary 3.2.2. *If the xy-direction has no cut elements, then there are at least two internally disjoint dipaths from x to y.*

Proof. If there are no cut elements, then in particular the minimum number of vertices whose deletion renders the xy-direction disconnected has to be at least two. Therefore, according to the theorem, the maximum number of dipaths internally disjoint from x to y is at least two, as well. □

The above corollary is the core idea that we will use for reformulating the Fiedler-Mochizuki Theorem 2.2.1 in a way that does not involve dipaths. In fact, it asserts the existence of internally disjoint directed paths without showing them concretely. According to our intuition, which says that the difficulty of proving transitivity of Theorem 2.2.1 relies on the dipaths formulation, it is then clear why this may be a very important step.

3.3 Obliged elements

In this section we want to introduce the main tools of our work. Although their meaning and their power is in a sense strictly connected with monomolecular reaction networks, we introduce them in a more general and abstract setting. Indeed, we have found no particular constriction to apply them just to this particular kind of networks. We refer simply, in this occasion, to a directed graph. The only exception is Proposition 3.3.6, which we have already set in a monomolecular reaction network environment, since it is particularly linked with the next proof of transitivity of the Fiedler-Mochizuki Theorem 2.2.1.

3.3.1 Definition

Definition 3.4 (Obliged Elements)**.** Let Γ be a digraph. For every x, y vertices such that y is reachable from x we define O_y^x (*obliged elements from x to y*) as a subset of elements of the xy-direction such that

$$O_y^x = \bigcap^{ALL} \{\gamma(x,y)\}$$

where $\gamma(x,y)$ is any dipath which starts at x and ends at y, but does not include the starting vertex x and the ending vertex y.
A single *obliged element to y* is therefore in particular a single element of Γ, either a vertex or an arrow.
We extend our definition for the arrow case recursively; for every x vertex, and i arrow reachable from x with tail $y = t(i)$, we define $O_i^x := O_y^x$.

We are now interested in using this tool for defining some kind of order knowledge, which is helpful for further purposes. This is, for many reasons, not a trivial issue. What we have found useful is to define what does it mean, for an element, to be after another element. The following definition explains precisely the meaning of this previous sentence:

Definition 3.5. Let p, q be elements in a directed graph, both reachable from a vertex x. The element q is said to be *x-after p* if p is an obliged element from x to q, i.e., $p \in O_q^x$. Moreover, we consider any element reachable from x to be x-after x.

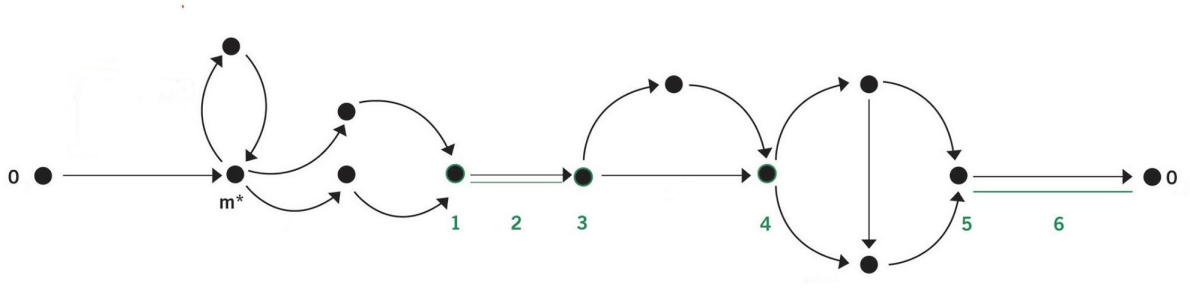


Figure 3.5: *In a generic monomolecular reaction network, we have selected in green the obliged elements from m^* to 0. In this case it is particularly easy to see that these are also the cut elements of the m^*0-direction, cfr: Propostion* 3.3.2*. It is also clear that they possess a certain order in the sense specified by Proposition* 3.3.1*.*

3.3.2 Propositions

With x, y vertices as above, let $\gamma = \gamma[x, y]$ be a dipath from x to y. Trivially, γ induces a natural order in O_y^x, the obliged elements from x to y, since all the obliged elements are in particular elements of γ, which, as a dipath, possesses a natural order. The following proposition asserts that all the dipaths from x to y agree on this order.

Proposition 3.3.1. *Any dipath $\gamma = \gamma[x, y]$ from x to y induces the same order in the set O_y^x of the obliged elements from x to y and therefore O_y^x is a totally ordered set.*

Proof. Assume indirectly that there exist $\bar{\gamma}, \tilde{\gamma}$ dipaths from x to y that induce in the set O_y^x a different order, i.e.,
$\exists\, p, q \in O_y^x$, obliged elements from x to y such that:
$p < q$ in the order given by $\bar{\gamma}$;
$q < p$ in the order given by $\tilde{\gamma}$.
Consider now the last-minute shortcut $\gamma^* = s(\bar{\gamma}[x, p]\tilde{\gamma}[p, y])$: γ^* is a dipath from x to y, but γ^* does not contain q. Indeed neither $\bar{\gamma}[x, p)$ nor $\tilde{\gamma}[p, 0]$ contains q since $\bar{\gamma}$ reaches p before q and $\tilde{\gamma}$ reaches p after q. Hence q is not an obliged element against our hypothesis and we have a contradiction.

□

Observation 7. As a first consequence, with the above settings, since the two dipaths $\bar{\gamma}$ and $\tilde{\gamma}$ agree on the order of O_y^x, in particular we have that, if p is an obliged element from x to y,

$$s(\bar{\gamma}[x, p]\tilde{\gamma}[p, y]) = \bar{\gamma}[x, p)\tilde{\gamma}[p, y].$$

Indeed, according to the above proposition, the last intersection between $\bar{\gamma}[x, p]$ and $\tilde{\gamma}[p, y]$ has to be p.

We now observe an alternative way to look at the obliged elements.

Proposition 3.3.2 (equivalent definition of Obliged Elements)**.** *Let p be reachable from x, and let the set of the obliged elements from x to p be nonempty, then the xp-direction is not 2-connected and the following statements are equivalent:*

1. *q is an obliged element from x to p;*
2. *q is a cut element of the xp-direction.*

Proof. If O_p^x happens to be nonempty, then for every element q in O_p^x, any dipath from x to p has to contain q. If we remove q from the digraph, then there is no possibility for reaching p from x with a dipath: namely p is not reachable from x anymore. On the other hand, p is not reachable anymore also with an undirected path. For seeing this, we should focus on the fact that any element of the xp-direction has to lie on a dipath from x to p. Assuming indirectly that it is possible to have an undirected path ψ from x to p, namely a sequence of vertices and edges, it is easy to reach a contradiction: the sequence indeed, according to the construction of the xp-direction with a source x and a sink p, has to begin following the order, and to end following the order. Therefore, in particular, the first element of the sequence is not x-after q, and the last one is for sure x-after q. This implies, in particular, that in our undirected dipath there is at least one

vertex y, which is not x-after q, followed by an undirected edge j^{-1}, which is x-after q. Clearly, in particular j^{-1} is an ingoing arrow of y, namely it is one arrow which does not follow the order. Now it is enough to consider a dipath γ_1 containing x and a dipath γ_2 containing j for obtaining a dipath $\gamma_p{:=}\gamma_1[x,y)\gamma_2[y,p]$ from x to p not containing q, which is a contradiction. In conclusion, p is not reachable anymore from x with an undirected path and therefore, by definition of 2-connectivity, the xp-direction is not 2-connected and the two statements are equivalent. □

In the following chapters we will give further equivalent formulations of the above concepts. Of course we might have chosen just one formulation for ever, but we have decided to keep all of them for giving the reader a wider range of options to think at these ideas. It is indeed our opinion that for certain concepts it works better one formulation, and for other concepts another formulation.

Let now x, y be vertices such that y is reachable from x, and let j be an outgoing arrow from x. We state also an equivalent characterization for j of being in the xy-direction.

Proposition 3.3.3. *Let x, y be vertices such that y is reachable from x, and j an outgoing arrow of x. The following statements are equivalent:*

1. *j is not in the xy-direction;*
2. *for every vertex z in $R^{x,j}$ such that y is reachable from z, the vertex x is an obliged element from z to y.*

[We recall that $R^{x,j}$ consists in all the elements of the graph reachable from x with a directed path starting along j.]

Proof. 1) ⇒ 2)
Assume indirectly that x is not an obliged element from z to y.
Then there exists a dipath γ_y^z from z to y s.t. $x \notin \gamma_y^z$. We can therefore consider the last minute shortcut $\bar{\gamma}{=}s(\gamma_z^x\gamma_y^z)$ where γ_z^x is any dipath from x to z containing j.
$\bar{\gamma}$ is then a dipath from x to y, and it contains j since γ_y^z does not contain x, the tail of j, and hence in particular the last intersection between the two dipaths in the order of γ_y^z (according with the definition) cannot be x. So we have reached a contradiction.
2) ⇒ 1)
Consider any diwalk $[xj...y]$ from x to y with j as first edge.
Assume now that it is possible to write some of these diwalks as a concatenation of two *dipaths* $[xj...z][z...y]$. Otherwise trivially j is not in the xy-direction, since any dipath can be written as a concatenation of two dipaths.
So we have that z is in $R^{x,j}$ and therefore, by our assumption, $x \in O_y^z$.
This holds for every z vertex in the j-subgraph and therefore every diwalk $[xj...y]$ counts x at least twice, namely no diwalk is a dipath. Hence j is not in the xy-direction.
□

Corollary 3.3.4. *In particular, we have that if j is not in the xy-direction the intersection between $R^{x,j}$ and the xy-direction is just x.*

Proof. Assume indirectly that $\exists p \neq x$ such that $p \in R^{x,j} \cap xy$-direction. Then, trivially, x is not an obliged element from p to y if p is a vertex, or from $h(p)$, the head of p, to y if p is an arrow. Indeed, by definition of xy-direction, p lies in a directed path γ from x

to y. By definition of directed path, considering the subpath $\tilde{\gamma} = \gamma[p, y]$, we have that $\tilde{\gamma}$ does not contain x. This leads to a contradiction with the above proposition. □

Despite its deceptive simplicity, the following Lemma is a crucial point of all our work. It is the link with Menger's Theorem and it is at the core of the theorems in the following chapters. We recall that two directed paths from vertex x to vertex y are internally disjoint if they share just the starting point x and the ending point y and no other elements.

Lemma 3.3.5. *Let x, y be vertices such that y is reachable from x. The followings are equivalent:*

1. *there are no obliged elements from x to y, i.e., $O_y^x = \emptyset$;*
2. *$\exists$ at least two dipaths $\gamma_{1,2} = \gamma_{1,2}[x, y]$ from x to y internally disjoint.*

Proof. 2) $\Rightarrow$ 1).
Indeed,

$$O_y^x = \bigcap^{ALL} \{\gamma(x, y)\} \subseteq \gamma_1(x, y) \cap \gamma_2(x, y) = \emptyset.$$

Again, we recall the exceptional notation of $\gamma_{1,2}(x, y)$ that is, in this case, simply $\gamma_{1,2}$ without the starting and ending vertices, namely without x and y.

1) $\Rightarrow$ 2):
This part is just the Corollary 3.2.2 of Menger's Theorem, since the obliged elements are precisely the *cut elements* of the xy-direction.

□

The following result, only, will assume the settings of the paper by Fiedler and Mochizuki [2]: namely, we consider a monomolecular reaction network, i.e., a strict directed graph with a special vertex 0 such that from any other vertex m there exists a dipath from m to 0. It is an inclusion result and it is another fundamental tool for the proof of transitivity of Theorem 2.2.1. In fact we may roughly say that inclusion relation and order relation are very good signs for a transitive relation.

Proposition 3.3.6. *Let γ^{m^*} be a dipath which starts at the metabolite m^*. Suppose that the path γ^{m^*} does not contain any obliged element from m^* to 0, i.e., $\gamma^{m^*} \cap O_0^{m^*} = \emptyset$. Then, for every vertex $\tilde{m}$ of γ^{m^*} the following inclusion holds:*

$$O_0^{m^*} \subseteq O_0^{\tilde{m}},$$

i.e., the obliged elements from m^* to 0 are contained in the ones from $\tilde{m}$ to 0.

Proof. Suppose indirectly that $\exists p \in O_0^{m^*}$ s.t. $p \notin O_0^{\tilde{m}}$.
Then, by definition of obliged elements from $\tilde{m}$ to 0, it exists dipath $\tilde{\gamma}_0 = \tilde{\gamma}_0[\tilde{m}, 0]$ s.t. p is not in $\tilde{\gamma}_0$.
We can therefore consider the last-minute shortcut $\bar{\gamma}_0 = s(\gamma^{m^*}[m^*, \tilde{m})\tilde{\gamma}_0)$, by construction $\bar{\gamma}_0$ is an exit dipath from m^* to 0 s.t. p is not in γ_0 against the hypothesis that p is an obliged element from m^* to 0. Hence we have a contradiction. □

Again, we would like to see the result from another point of view, in the sense explained by the following observations.

Observation 8. The above proposition can be identically reformulated, in a more abstract way, as follows: let m^* be a vertex and x the first obliged element from m^* to 0, for every metabolite $\tilde{m}$ reachable from m^*, not m^*-after x, the following holds:

$$O_0^{m^*} \subseteq O_0^{\tilde{m}}$$

Observation 9. In particular then, since every obliged element p from m^* to 0 is an obliged element from every $\tilde{m}$ not m^*-after x first obliged element, it exists a dipath from $\tilde{m}$ to p. Of course, this is trivial but we find useful to point out also some trivial features, since it is different to understand one single triviality or to keep track and recollect ten of those.

Let us conclude by giving a very intuitive and not mathematical explanation of the last proposition: imagine we are in a room that has a storage room reachable just with a door from the room where we are. If we go wherever without passing this door, and then want to reach the storage room, we will eventually have to pass though this single door. This is the precise meaning, in a directed graph contest, of the above proposition.

Chapter 4

Reformulation of the flux-influence theorem and the transitivity proof

First of all, we make a little modification to the settings of the theorem. We recall that the third assumption of the Fiedler-Mochizuki Theorem 2.2.1 on flux response is the regularity of the network at the steady state x^*. From [2] we know that this assumption implies directly, from a graph point of view, that for every metabolites m there exists a dipath starting from m and reaching 0. On the other hand, for the chemical meaning of the zero complex, it has to be always possible to reach any metabolites from 0.
This means in particular that for any couple m_1, m_2 of metabolites at least two dipaths γ_1 and γ_2 exist joining the two vertices in both direction, i.e., such that

$$\gamma_1 = \gamma_1[m_1, m_2]$$

$$\gamma_2 = \gamma_2[m_2, m_1]$$

This is easy to see, just by considering the exit directed paths from m_1 and m_2 to 0 and, let us say, the *feed* directed path from 0 to m_1 and m_2.
Therefore, any couple of metabolites lies on a dicycle, and in particular any metabolite is reachable from any other and we have the fact that all the network is reachable from m_1 and reachable from m_2. More generally, for any metabolite m, R^m, namely the subgraph reachable from m, coincides with the whole network. This fact is unfortunate for our purposes, since it clearly renders less useful the tools we have defined in the preceding chapters. On the other hand it is not of chemical relevance, since passing 'through' 0 means exactly to nullify the reaction. Therefore, for these further notes on a graph environment only, we consider the 0 vertex to be split onto two different vertices: 0_F and 0_E, where 0_F has just all the outgoing arrows, namely all the feed reactions, of the original 0 and 0_E has all the ingoing arrows, namely all the exit reactions.
All the consequences of the theorem still hold trivially. For not making the notation too much involved, we will avoid to add the pedices $_F$ and $_E$ to the 0. No ambiguity will arise, however, since a dipath leading to 0 in the former settings will now leads to 0_E (the 0 vertex with just all the ingoing arrows of the original 0), and a dipath emanating from 0 in the former settings will now emanate from 0_F. The special easy cases in the theorem, in which at least one, between m^* and m' (tail of j'), is 0, have to be treated again with the simple rule that both 0_F and 0_E are 0 vertices. Basically we consider the original '0-vertex' to be actually a set of two vertices. It is straightforward to see that nothing changes in the conclusions of the theorem.
Let us do just one easy example: a simple consequence of Theorem 2.2.1 is that if j is an

outgoing arrow from 0, then j influences any arrow reaction in $R^{0,j}$, namely any reaction reachable from 0 with a dipath starting along j. This can be seen, considering γ^0 of the theorem to be the empty dipath $\gamma^0 = \{0\}$. We see now, with the new settings, the situation as follows: j is an outgoing arrow from 0_F. Again j influences any reaction in $R^{0_F,j}$, since we consider again simply the empty path $\gamma^0 = \{0_F\}$.

The leading idea for the following reformulation, we repeat, is to get rid of the γ^0, dipath from the m^* tail of the perturbed reaction j^* and reaching 0, obtaining a statement without it. This will be of great relevance and use for proving in an easier way the transitivity of the theorem itself.
One might be tempted to address a reformulation of the theorem with no dipaths at all. This will be done, in a more general sense, in Chapter 5, where we characterize the whole set of reactions (arrows) influenced by a perturbation of a single one. It is easier, nevertheless, doing it with the following reformulation of the theorem as a first step. Indeed, after having done it and used it, it will be straightforward to make statements, even more general, without having to regard at all directed paths.

4.1 Reformulation of the theorem

We refer to the conditions of the original theorem with i) ii) iii) iv), and to the objects of the reformulated conditions with a subscript $_R$.

Theorem 4.1.1 (Reformulated conditions)**.** *The four conditions in the Fiedler-Mochizuki Theorem* 2.2.1 *on the Flux response hold true if, and only if, there exists a directed path γ'_R such that the following three conditions all hold true:*

1. *γ'_R emanates from vertex m^* and terminates with reaction edge j', not including the head vertex of j', i.e., $\gamma'_R = \gamma'_R[m^*, j']$,*
2. *γ'_R has empty intersection with the obliged elements from m^* to 0, i.e., $\gamma'_R \cap O_0^{m^*} = \emptyset$,*
3. *j^* is in the m^*0-direction or it is in γ'_R (both may happen simultaneously).*

Before going on to prove the theorem, we explain the idea of the proof. Reading the complete and precise proof might be indeed far more difficult than understanding what is going on. The fact that the old formulation implies the new is very easy to prove. The opposite direction, however, that the new formulation implies the old, is more complicated. For doing this we will have indeed to build two directed paths as the old Fiedler-Mochizuki theorem requires. Except from the easy situations, what we generally need to show is that condition 2 of the new reformulated theorem implies the existence of two disjoint paths. We use then Lemma 3.3.4 which guarantees us that two internally disjoint paths exist between m^* and the first obliged element from m^* to 0. This provides us an undirected cycle structure with a source (m^*) and a sink (the first obliged element from m^* to 0). Then we intersecate this structure with γ'_R for building the two desired directed paths.

Proof. We start with the easy part: the conditions of Theorem 2.2.1 imply the reformulated conditions 1), 2) and 3).

Indeed, let γ'_R:=γ'
Condition 1) of the reformulated conditions is verified by definition of γ'.
Condition 2) can be proved indirectly: if $\gamma'_R \cap O_0^{m^*} \neq \emptyset$ then there not exists an exit dipath γ_0 with empty intersection with γ' as in Theorem 2.2.1, since any exit dipath γ_0 contains all the obliged elements from m^* to 0.
The last condition 3) of the new theorem is directly implied by condition ii) in Theorem 2.2.1.

Assume now the reformulated conditions 1, 2 and 3. Our goal is to show that there exist two directed paths γ_0 and γ' as in the Fiedler-Mochizuki Theorem 2.2.1.

Let us firstly note that j^* cannot be the only outgoing arrow from m^*. If this would be the case, indeed, j^* would be in particular the first obliged element from m^* to 0 and there would not exist a directed path γ'_R from m^* *not* containing the obliged element j^*, contradicting the reformulated conditions.

Moreover, if j^* is the only outgoing arrow from m^* in the m^*0-direction, in particular j^* is the first obliged element from m^* to 0. Then the dipath γ'_R emanates from m^* along an arrow $i \neq j^*$ which is *not* in the m^*0-direction. In particular, according to Corollary 3.3.4, any exit dipath γ_0 from m^* to 0, belonging to the m^*0-direction, does not intersect γ'_R, belonging to $R^{m^*,i}$. Hence we have that the pair of directed paths so defined (γ_0, γ'_R) easily fullfills the conditions i), ii), iii), iv) of Theorem 2.2.1.

Let us, therefore, now assume that m^* has at least two outgoing arrows in the m^*0-direction, then the first obliged element from m^* to 0 is a vertex x. It might happen also that there are no obliged elements from m^* to 0, in this case we set simply $x := 0$. We have, trivially, that the set of obliged elements from m^* to x, $O_x^{m^*}$, is empty. We can therefore apply Lemma 3.3.5 and we obtain two dipaths $\gamma_{1,2}$ from m^* to x internally disjoint, namely that they do intersect just in m^* and x, i.e. $\gamma_1 \cap \gamma_2 = \{m^*, x\}$.
We now want to use the structure of this undirected cycle of $\gamma_1 \cup \gamma_2$. We have to check all possible cases.
The case in which γ'_R intersects $\gamma_1 \cup \gamma_2$ just in m^* is easier. Indeed we take γ'_R and $\tilde{\gamma_0} := \gamma_1[m^*, x)\gamma_x[x, 0]$, where γ_x is any exit dipath from x to 0. As already noticed, we have that γ'_R and $\tilde{\gamma_0}$ do intersect *only* in m^*. If they intersected in a vertex $w \neq m^*$, indeed, in particular $w \in \gamma_x$ since γ'_R intersect γ_1 just in m^*. We may consider, then, the sequence $\gamma'_R[m^*, w)\gamma_x[w, 0]$ obtaining a directed walk from m^* to 0 not containing the obliged element x, which is a contradiction. Therefore these two directed paths, γ'_R and $\tilde{\gamma_0}$ satisfy conditions i), iii), iv) of Theorem 2.2.1. Moreover, if j^* is not in the m^*0-direction, then it has to be in γ'_R and we have also condition ii).
If γ'_R has more than one intersection with $\gamma_1 \cup \gamma_2$, in particular we have that j^* is in the m^*0-direction, since we can build easily, with a last-minute shortcut, a dipath from m^* to 0 with j^* as first arrow.
Let y be the last element, in the order given by γ'_R, of $[\gamma_1 \cup \gamma_2] \cap \gamma'_R$. We know for sure that it is not x by condition 2. Without losing our generalities, we assume $y \in \gamma_1$. We can obtain a new dipath from m^* to j' by

$$\tilde{\gamma'} := \gamma_1[m^*, y)\gamma'_R[y, j']$$

By construction, we have that $\gamma_2 \cap \tilde{\gamma'} = \{m^*\}$, namely our new dipath from $\tilde{\gamma'}$ still does

not intersect γ_2 apart from in m^*.
Let γ_x be any exit dipath from x to 0. The two dipaths $\tilde{\gamma}_0 = \gamma_2\gamma_x$ and $\tilde{\gamma}'$ already satisfy conditions i), iii), iv).
In any unfinished situation, we have at least found a pair of directed paths $(\tilde{\gamma}_0, \gamma')$ such that the conditions i), iii), iv) of Theorem 2.2.1. We need now to work on condition ii): j^* can, indeed, belong to $\tilde{\gamma}'$, $\tilde{\gamma}_0$ or to none. In the first two cases, we have for free also condition ii), hence we have finished.
In the third case: $j^* \notin \tilde{\gamma}_0 \cup \tilde{\gamma}'$, but j^* has to belong to some γ_0^* exit dipath from m^*, since we have already noticed that j^* is in the m^*0-direction.
Let z be now the *first* element after m^* of $[\tilde{\gamma}_0 \cup \tilde{\gamma}'] \cap \gamma_0^*$, in the order given by γ_0^*. (For sure the intersection is not just m^* since also x surely belongs to this intersection).
Just the followings can happen:
1) $z \in \tilde{\gamma}_0 \qquad \gamma_0 := \gamma_0^*[m^*, z)\tilde{\gamma}_0[z, 0] \qquad \gamma' := \tilde{\gamma}'$
2) $z \in \tilde{\gamma}' \qquad \gamma' := \gamma_0^*[m^*, z)\tilde{\gamma}'[z, j'] \qquad \gamma_0 := \tilde{\gamma}_0$
Considering the couple (γ_0, γ') as above defined we easily verify that it satisfies conditions i) ii) iii) iv). □

We can now, of course, reformulate the Fiedler-Mochizuki Theorem 2.2.1 on the Flux-influence as follows:

Theorem 4.1.2 (Reformulated theorem)**.** *Let the above assumptions hold, and consider any pair of reaction arrows (j', j^*), not necessarily distinct.*
Then j^* flux-influences j' ($j^* \rightsquigarrow j'$) if, and only if, there exists a directed path γ'_R s.t. the followings all hold true:

1. *γ'_R emanates from vertex m^* and terminates with reaction edge j', not including the head vertex of j', i.e. $\gamma'_R = \gamma'_R[m^*, j']$,*
2. *γ'_R has empty intersection with the* obliged elements from m^* to 0*, i.e. $\gamma'_R \cap O_0^{m^*} = \emptyset$,*
3. *j^* is in the m^*0-direction or it is in γ'_R (both may happen simultaneously).*

4.2 Transitivity

We have finally reached our initial aim: to state and prove in a good way a transitivity result for the flux-influence relation.

Theorem 4.2.1. *The flux-influence relation is a transitive relation, i.e.,*

$$j^* \rightsquigarrow j' \rightsquigarrow j'' \text{ implies } j^* \rightsquigarrow j''.$$

Proof. Let's call the three vertices with relative arrows and dipaths $m^*, j^*, \gamma', m', j', \gamma'', m'', j''$.
Here:
m^* is a metabolite and j^* is an outgoing arrow from m^*.
m' is a metabolite and j' is an outgoing arrow from m'.
m'' is a metabolite and j'' is an outgoing arrow from m''.
γ' is a directed path from m^* to j' as in the reformulated theorem, namely such that j^* flux-influences j'.
γ'' is a directed path from m' to j'' as in the reformulated theorem, namely such that j'

flux-influences j''.

We therefore may assume that the three conditions of the reformulated theorem hold for the couple (j^*, j') and for (j', j''). We have to show that they also hold for the pair (j^*, j''), so that j^* flux-influences j''.

1. We have to show that a directed path exists from m^* to j''. For doing this it is enough to consider the last-minute shortcut from $s(\gamma'[m^*, m']\gamma'')$. Let's call this path γ^2. By construction $\gamma^2 = \gamma^2[m^*, j'']$, namely a directed path from m^* to j''.

2. We have to show that our γ^2 has empty intersection with the obliged elements from m^* to 0.
   By Proposition 3.3.6,
   $$O_0^{m^*} \subseteq O_0^{m'}$$
   Indeed, by the fact that j^* influences j', we have in particular that m' is reachable from m^* with a directed path which does not include any of the obliged element from m^* to 0. That is $\gamma' \cap O_0^{m^*} = \emptyset$.
   On the other hand, since j' influences j'', we have, by condition 2 of the reformulated theorem, that $\gamma'' \cap O_0^{m'} = \emptyset$.
   So we obtain that in particular $\gamma'' \cap O_0^{m^*} = \emptyset$.
   Therefore, we can conclude that the intersection between the directed path γ^2 from m^* to j'' and the obliged elements from m^* to 0 is empty. γ^2 is indeed made up by γ' and γ'' which have both empty intersection with that set. Technically:
   $$[\gamma^2 \cap O_0^{m^*}] \subseteq (\gamma'[m^*, m')\gamma'') \cap O_0^{m^*} = (\gamma'[m^*, m'] \cap O_0^{m^*}) \cup (\gamma'' \cap O_0^{m^*}) = \emptyset$$
   Hence we have obtained that $[\gamma^2 \cap O_0^{m^*}] = \emptyset$

3. We have to show that either j^* is in the m^*0-direction or j^* is in γ^2.
   If j^* is in the m^*0-direction we have finished: our directed path γ^2 fits indeed completely the conditions of the reformulated theorem.
   If j^* is not in the m^*0-direction, then j^* belongs to γ', hence m' is in $R^{(m^*,j)}$, the subgraph reachable from m^* with a directed path starting along j^*. To conclude that j^* is in γ^2 we just need to check that j^* is not a skipped element of the last-minute shortcut $s(\gamma'[m^*, m')\gamma'')$. Since j^* is the first element after the starting point m^* of γ', the only possibility for j^* to be a skipped element is that m^* itself is the last intersection between γ' and γ'' in the order given by γ''. For this to happen, in particular, m^* has to be in γ''. But, by Proposition 3.3.3, we know that m^* is an obliged element to 0 for any vertex in R^{m^*,j^*}, in particular for m'. Therefore γ'' does not contain m^*, by condition 2 of the reformulated theorem. Hence j^* is not a skipped element of the shortcut and it belongs to γ^2.

□

Chapter 5

Characterization of the influenced sets

Now it is also possible to easily derive a characterization for the whole set of the influenced reaction arrows of j^*. We indicate the set of arrows influenced by j with $I(j)$ and the subgraph made by $I(j)$ together with any tail vertex of the arrows in $I(j)$ as $\bar{I}(j)$.
First of all, we make an observation: in these networks, from any metabolites a dipath to 0 always exists. Then, of course, any metabolites m has at least one outgoing arrow j_0 such that j_0 is in the $m0$-direction. In particular, therefore, let m be a metabolite and j an outgoing arrow from m. If j is *not* in the $m0$-direction, then m has at least another outgoing arrow j_0 different from j.

5.1 Main result

As announced in the previous chapter, the following theorem provides a formulation without dipaths in its statement.

Theorem 5.1.1. *Let j^* be a reaction arrow in a monomolecular reaction network. Let $m^* = t(j^*)$ be its tail.*

1. *If j^* is in the m^*0-direction,*
   then $I(j^*)$ consists of all the arrows reachable from m^* not m^*-after the first obliged element from m^* to 0.

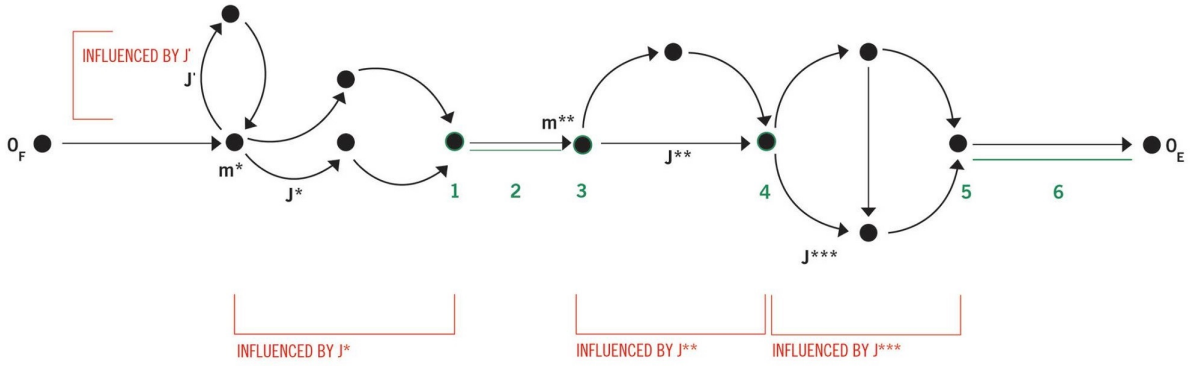
2. *If j^* is not in the m^*0-direction,*
   then $I(j^*)$ consists of all the arrows in R^{m^*,j^*}, namely all the arrows reachable from m^* with a directed path starting along j^*.

Proof. Using the reformulated theorem:
Part 1: if j^* is in the m^*0-direction, condition 3 of the reformulated theorem is always satisfied. Therefore we just need to take care of the fact that j' is reachable with a directed path with empty intersection with the obliged elements from m^* to 0, namely conditions 1-2 of the theorem. By the total order of the set of the obliged elements, this condition turns to hold just if j' is not m^*-after the first of them.
Part 2: if j^* is not in the m^*0-direction, then, by condition 3, j^* has to be in γ'_R. Therefore to be in R^{m^*,j^*} is a necessary condition. By Proposition 3.3.3 we have that this condition

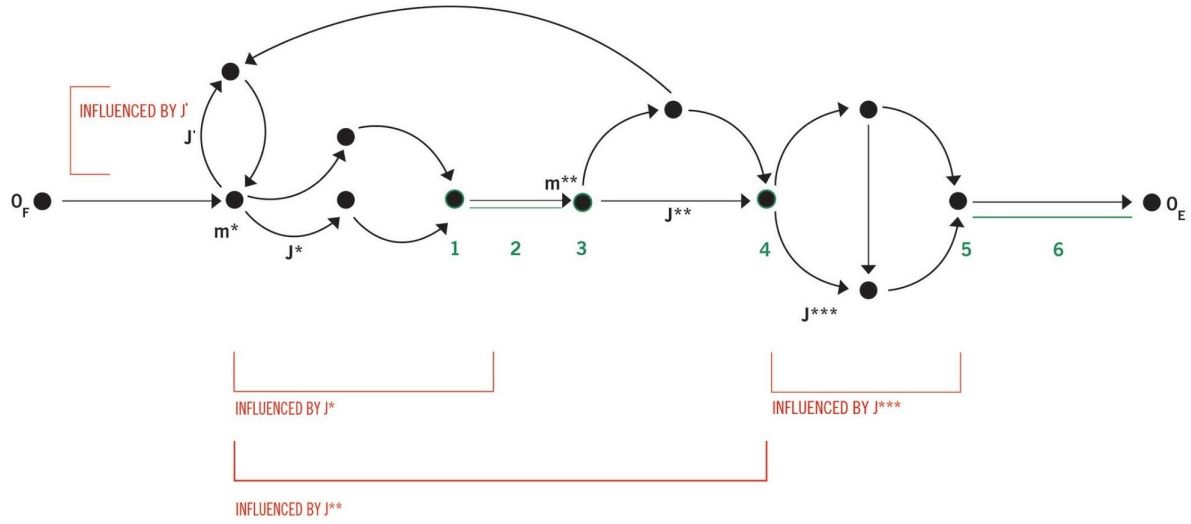
is also sufficient, since no exit dipath from m^* to 0 intersects with R^{m^*,j^*} if j^* is not in the m^*0-direction, apart trivially in m^*. In particular, no obliged element lies in R^{m^*,j^*}. □

We underline the fact that, in particular, the influenced set of any arrow j^* outgoing from a metabolite m^* is at most given by all the arrows reachable from m^* not m^*-after the first obliged element from m^* to 0, no matter whether it is in the m^*0-direction or not. Indeed every arrow reachable from m^* with a directed path starting along an arrow which is not on the m^*0-direction trivially does not intersect, at all, any obliged element from m^* to 0. This is, in a sense, a localization result, which we will deepen in the next chapter.

Using again the example in Figure 3.5, we apply our result to this case.



We have chosen four arrows: J^*, J^{**}, J^{***} and J'. Only J' is not in the m^*0-direction, where m^* is the mother of J' and J^*. In this example we want particularly to point out the link between the obliged elements and the influenced set. We see indeed that the different influenced sets are exactly the 2-connected components of the graph. This holds in this precise way here because this is a particular example, which has been chosen for this purpose. Nevertheless the idea is the same and also applies to more complicated examples.
Note that the set influenced by J' is a subset of the one influenced by J^* since J^* is in the m^*0-direction while J' is not.
Looking at this figure, we may be tempted to conclude that the obliged elements from 0_F to 0_E divide the networks influenced components which do not interact between each other. This is only partially true. In the next example we have added to the network just a simple reaction arrow and the situation changes significantly.
The set influenced by J^{**}, $I(J^{**})$, now contains the one influenced by J^*. This happens, according to our theorem, because J^{**} influences all the arrows not m^{**}-after the first obliged element from m^{**} to 0. This suggests, correctly, that the important thing for a reaction to influence the largest amount of reactions is not to be close to the feed 0_F and namely to be one of the first reaction which happens in the network, but rather to be possibly far away from the exit 0. In this last example, indeed, J^* is the very first reaction (together with J' and the other outgoing from m^*) right after the $feed$ one. But J^{**} is farther from exit 0_E in the sense that J^* is reachable from J^{**}



5.2 Transitivity-inclusion result

Although it is already implicitly proven by the transitivity theorem in the previous chapter, we derive now an analogous result of transitivity from the above theorem.
In doing this, let us observe that if x, y are vertices in a network Γ such that y is reachable from x, then the subgraph $R^y(\Gamma)$ reachable from y is contained in $R^x(\Gamma)$ reachable from x, namely $R^y \subseteq R^x$ if $y \in R^x$. Indeed, for any vertex y reachable from x it exists a dipath γ_y^x from x to y. For any vertex z reachable from y analogously exists a dipath γ_z^y from y to z. Then it is enough to consider the last-minute shortcut $s(\gamma_y^x\gamma_z^y)$ for concluding that any vertex reachable from y is also reachable from x.
Let us observe moreover that if y is reachable from x with a directed path starting along j, outgoing arrow from x, then any element reachable from y with a directed path, which does not contain x, is again reachable from x with a directed path starting along j. In seeing this, it is enough to consider a last-minute shortcut. As in part 3 of the proof of transitivity in the last chapter, here necessary condition for j to be a skipped element is that x is in the intersection of the two directed paths, fact that we have excluded.

Let j^*, outgoing arrow from m^*, be in the m^*0-direction, and let j' be any arrow reachable from m^* with a directed path not intersecting the first obliged element x from m^* to 0. We have then the following conclusions:

1. j' is influenced by j^*.

2. x is an obliged element also from $m' = t(j')$ to 0, according to Proposition 3.3.6.

Then, by using the above observation, we conclude easily that the influenced set of j' is included in the influenced set of j^*. Indeed, both if j' is in the $m'0$-direction or not, all the arrows reachable from m' not m'-after the first obliged element from m' to 0 are reachable from m^* and are not m^*-after the first obliged element from m^* to 0.
If j^* is *not* in the m^*0-direction, then for any $\tilde{m}$ in R^{m^*,j^*} reachable from m^* with a directed path starting along j^*, the influenced set of an outgoing arrow $\tilde{j}$ of $\tilde{m}$ is *at most* any arrow i reachable from $\tilde{m}$ not $\tilde{m}$-after m^*, since for sure m^* is an obliged element

from $\tilde{m}$ to 0, again by Proposition 3.3.5. Although it might not be the very first. Any arrow of this kind, in particular, belongs again to R^{m^*,j^*} according to our observation above.

Hence we have proved (again) the following:

Theorem 5.2.1 (Inclusion)**.** *If a reaction j^* flux-influences a reaction j', then*

$$I(j') \subseteq I(j^*).$$

It is interesting to note that in this case, although we have spoken up to now about a transitivity result, it is more like an inclusion result. This leads directly to further questions, which are worth to mention, but will not be fully addressed in this thesis.
In particular an inclusion relation makes us think about a pyramid structure in which we would like to understand which are the bottom elements, namely the reactions which do not influence anything, and which are the top elements, namely the relations which influence the most. The first question will be addressed and easily solved in Chapter 7, it is indeed an easy consequence of previous results. The other direction is not as easy, some ideas may again be found in Chapter 7, but there is still much work to be done.

Chapter 6

Another point of view: Balloons

At the 2015 Equadiff Conference in Lyon, Hiroshi Matano gave a very interesting talk: *'Balloons in chemical reaction networks'*. His talk was extremely analogous to the topic we have presented in previous chapters. After inspiring discussions, it is our intention to present here briefly also his point of view, and to try to glue together our results.

Let Γ be our monomolecular reaction network, namely a strict directed graph which possesses a special vertex, the vertex 0_E, such that from any other vertex m there exists a directed path from m to 0_E.

We recall again Definition 3.2:

Definition 6.1 (Reachable Set)**.** Let m be a metabolite of a monomolecular reaction network Γ and j an outgoing arrow from m.
We define the reachable set $R^m(\Gamma)$ from m, as the subset of the network Γ, which consists in all the elements reachable with a directed path from m.
Moreover, we define the reachable set $R^{m,j}(\Gamma)$ from m, starting along j, as the subset of the network Γ, which consists in all the elements reachable with a directed path from m that has j as first arrow.

In this sense, as pointed out in Proposition 3.3.2, the obliged elements from any metabolite m to 0 can be identically defined precisely as the elements whose single deletion renders 0 not reachable anymore from m. Namely

$$O_0^m := \{p \in \Gamma \; s.t. \; 0 \notin R^m(\Gamma \setminus p)\}.$$

We recall again that this set possesses a natural total order, according to Proposition 3.3.1.

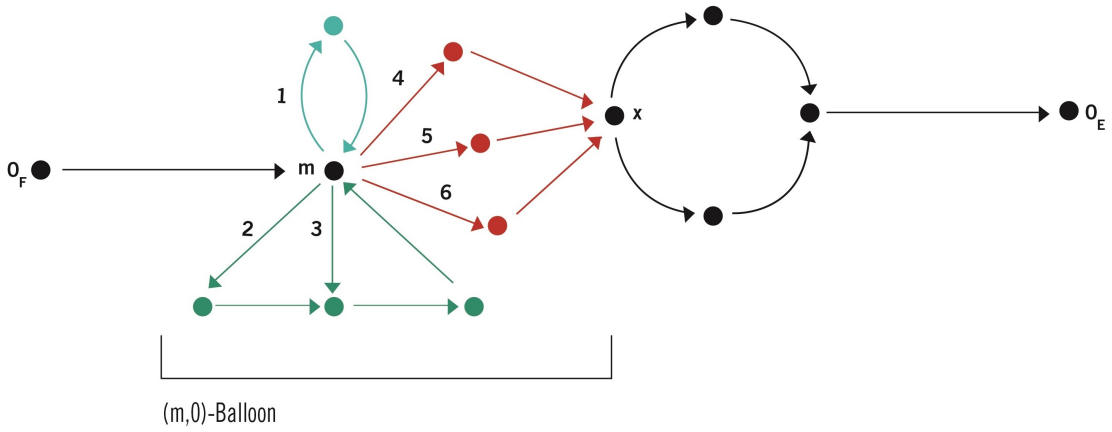
We define now the following subset of Γ:

Definition 6.2 (Balloon)**.** Let Γ be a monomolecular reaction network and m a metabolite of Γ. Let x be the first obliged element from m to 0. We define the $(m, 0)$-Balloon B_0^m as the subgraph of Γ reachable from m with a dipath without passing through x, the first obliged element from m^* to 0.

We may say that a Balloon consists of different components: for any outgoing arrow j' from m that is not in the $m0$-direction then the reachable set $R^{m,j'}$ is a subset of the $(m, 0)$-Balloon. Here we recall that the $m0$-direction is the subset of Γ made by all the directed paths from m to 0. Moreover, if this metabolite m has just another outgoing

arrow j'' a part from j', this implies that j'' has to be in the $m0$-direction, by definition of monomolecular reaction networks. Then the arrow j'' is clearly the first obliged element and the $(m,0)$-Balloon is just $R^{m,j'}$. But, if m has more then two outgoing arrows then, for any outgoing reaction j' from m which is not in the $m0$-direction, $R^{m,j'}$ is a strict subset of the $(m,0)$-Balloon.
Recalling, from Theorem 5.1.2, that the set of reactions influenced by a single perturbation of reaction j^*, when j^* is in the m^*0-direction, consists of all the arrows reachable from m^* not m^*-after the first obliged element from m^* to 0, we clearly see that this set is precisely made by all the arrows contained in the $(m^*,0)$-Balloon. Indeed, for an arrow i, to be m^*-after the first obliged element x from m^* to 0 means, by definition, that x is an obliged element from m^* to i, and therefore i is not reachable from m^* without passing through x.

We now want to demonstrate our arguments with an example:



The metabolite m has six outgoing reactions, which for convenience we have called in this case simply {1,2,3,4,5,6}. Reactions {1,2,3} are not in the $m0$-direction. Reactions {4,5,6} are in the $m0$-direction. We can see x, the first obliged element from m to 0. The subgraph $R^{m,1}$ reachable from m with a directed path starting along reaction 1 is coloured in light blue. In green we have selected the component of the $(m,0)$-Balloon given by the union of the subgraphs $R^{m,2}$ and $R^{m,3}$, reachable from m with a directed path starting along, respectively, 2 and 3. These two subgraphs are of course not distinct, but in particular reaction $2 \notin R^{m,3}$ and reaction $3 \notin R^{m,2}$. In red we can see the part of the $(m,0)$-Balloon in the $m0$-direction.
It can be also clearly seen that any element in the $(m,0)$-Balloon is not m-after x, the first obliged element from m to 0.

An equivalent reformulation of Theorem 5.1.2 on the characterization of the influenced sets, using this language, is therefore:

Theorem 6.0.2. *Let j^* be an arrow and m^* be its tail.*
Then the influenced set by j^*, $I(j^*)$, is made as follows:

1. *if $0 \in R^{m^*,j^*}$, then $I(j^*)$ consists of the arrows in the balloon $B_0^{m^*}$, and no others,*

2. *if $0 \notin R^{m^*,j^*}$, then $I(j^*)$ consists of the arrows in the reachable set R^{m^*,j^*}, and no others.*

Applying this point of view on our example, we have that:

1. the set influenced by reaction 1 consists of the arrows in light blue;
2. the set influenced by reaction 2 consists of the arrows in green without reaction 3;
3. the set influenced by reaction 3 consists of the arrows in green without reaction 2 and the other only outgoing arrow from the head metabolite of reaction 2;
4. the set influenced by reactions 4, 5, 6 is the same and it is the whole $(m, 0)$-Balloon.

Chapter 7

Consequences

In this chapter we want to underline and focus some consequences of the theorems stated in Chapters 4, 5, and 6, which could be useful for application in real networks. Again, a lot of them may be regarded as straighforward results; but, as previously stated, we believe that it is totally meaningful to gather a lot of straightforward results together and discover that one single triviality is a triviality, but the sum of ten trivialities are an interesting thing. We have derived all these consequence as easy corollaries, according to the theorems in the previous chapters. Unless otherwise specified, we will assume that e, i, j, k are outgoing arrows from a metabolite m.

1. The influenced set of an arrow j is empty, namely $I(j) = \emptyset$, if and only if its tail metabolite m has just one outgoing arrow j.
   Indeed, if m has just one outgoing arrow j, then j is in particular the first obliged element from m to 0. Of course no reactions are reachable from m without passing through j since j is the only outgoing arrow of m, then $I(j) = \emptyset$.
   In the other direction, if m has at least two outgoing arrows j and j_1, then j may be either in the $m0$-direction or not. If j is in the $m0$-direction, then j influences for sure j_1. If j is not in the $m0$-direction, then j influences for sure j itself. In both cases, $I(j) \neq \emptyset$.

2. Assume now that the set O_0^m of the obliged elements from m to 0 is nonempty and that the first obliged element of m is not an outgoing arrow from m itself, namely m has at least two outgoing arrows in the $m0$-direction. Then the first obliged element is a vertex x which has at least two ingoing arrows. This is just the corollary to Menger's theorem.

3. If j is in the $m0$-direction, then j influences j itself if and only if j is not the first obliged element from m to 0, namely if there is another arrow $k \neq j$ outgoing arrow from m, which is in the $m0$-direction as well.

4. If j is not in the $m0$-direction, then j influences j itself.

5. If j and i are two outgoing arrows from m, both in the $m0$-direction, then

   $$I(j) = I(i).$$

   Indeed the influenced set of both is defined by the same Balloon.

6. If j and i are the two only outgoing arrows from m, then

$$I(j) = I(i).$$

Indeed: if they are both in the $m0$-direction, then we have the previous observation. If one of them, w.l.o.g. i, is not in the $m0$-direction, then j is the first obliged element from m to 0. Therefore $I(j)$, the $(m, 0)$-Balloon, is just the set $R^{m,i}$ reachable by m with a directed path starting along i, namely $I(i)$. Note however that, in this last case, j influences i while i does not influence j.

7. In conclusion, if a metabolites m has n outgoing arrows with $p \leq n-2$ not in the $m0$-direction, then the number of different influenced sets of the outgoing arrows is exactly $p+1$, one for each reaction not in the $m0$-direction, and the Balloon influenced by the reactions in the $m0$-direction, which, in particular, contains all the other influenced sets.

8. If e is an arrow reachable from m (not necessarily outgoing from m!) in the $m0$-direction, not m-after the first obliged element x from m to 0, then e lies on an undirected cycle. We underline that this does not exclude that e lies also on a dicycle.
In fact, it is enough to consider one dipath from m to x, containing e, and then, with an analougous argument as in the proof of the reformulated conditions, construct another dipath from m to x not including e. The union of these two dipaths provide the structure of an undirected cycle.

9. If k is an arrow reachable from m (not necessarily outgoing from m!) not in the $m0$-direction, then k lies on a dicycle. We underline again that this does not exclude that k lies also on an undirected cycle.
Indeed, we can consider a dipath γ_1 from m to k and the concatenated exit dipath γ_2 from the metabolite head of k, $n = h(k)$, to 0. Now, if we consider the last-minute shortcut $s(\gamma_1\gamma_2)$ from m to 0, k has to be a skipped element, since k is not in the $m0$-direction. As a skipped element, in particular, k has therefore to lie on a directed cycle.

10. Therefore, gathering the two observations above, it follows that any element in any Balloon lies on a cycle (that may be directed or undirected). Cfr as an example: figure in Chapter 6.

11. In the other direction, if an arrow lies on a cycle (that may be directed or undirected), then it lies on a Balloon. In particular, a necessary and sufficient condition for any reaction in the network, which is not a feed arrow, for being influenced, is to lie on a cycle. Indeed, to lie in a Balloon is a necessary and sufficient condition for being influenced.

12. If a reaction j does not lie on a directed or undirected cycle, j is not influenced by any arrow but by the feed arrow.

13. If j does not lie on a directed or undirected cycle, it is an obliged element from 0_F to 0_E. We underline, however, that the opposite direction is not true.

14. Let j do not lie on a directed or undirected cycle. Let m be its tail metabolite $m = t(j)$ and n its head metabolite, i.e., $n = h(j)$. Of course, we have in particular that j is the first obliged element from m to 0. Then, any arrow m-after j, namely reachable from n, is not influenced by and does not influence any arrow not m-after j. Hence we have split the networks in two different region of influence which do not relate with each other.

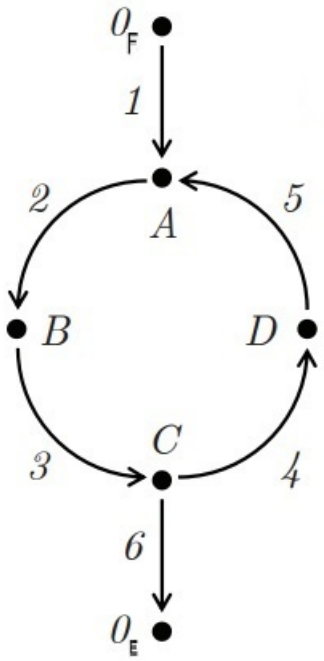
15. In particular, analysing the reactions that do not lie on a cycle, it may lead to the possibility of deconstructing the whole networks in smaller and independent networks.

Chapter 8

Examples

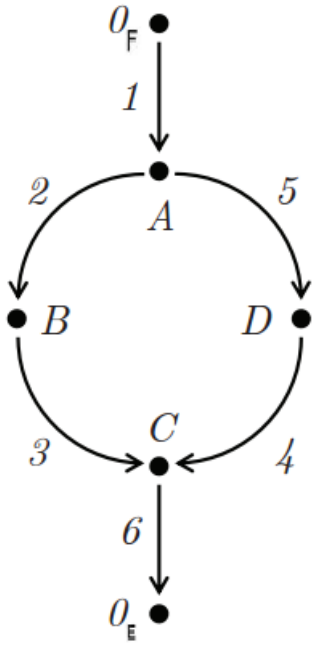
In this chapter we comment on examples taken from [2] by Fiedler and Mochizuki *Sensitivity of chemical reaction networks: a structural approach. 2. Regular monomolecular systems*. The figures are taken from [2] as well. We will point out some interesting features referring to [2] for the complete analysis of the flux-influence relation. In particular we will not focus, in our discussion, on the *feed* reactions, namely the outgoing reactions from the feed 0_F. This easy and specific case can be indeed treated all in the same easy way, as already done in [2].

Example I



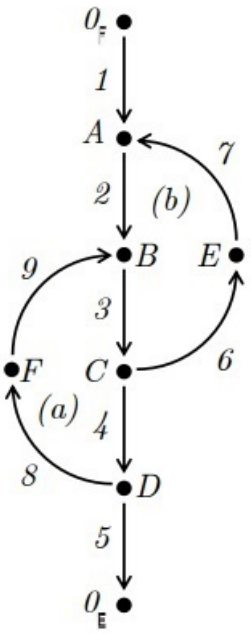
The obliged elements from 0_F to 0_E are *1, A, 2, B, 3, C, 6*. The only metabolite that has more than one outgoing reaction is C, which has reactions 6 and 4 as outgoing reactions. The reaction 6 is in the $C0$-direction, while reaction 4 is not in the $C0$-direction. According to our observations in the previous chapter, 4 and 6 influence the same set, which are all the reactions in the dicycle, namely reactions {2,3,4,5}. Reactions 5, 2 and 3 do not influence anything, as they are the only outgoing reaction from their mother metabolite.

Example II



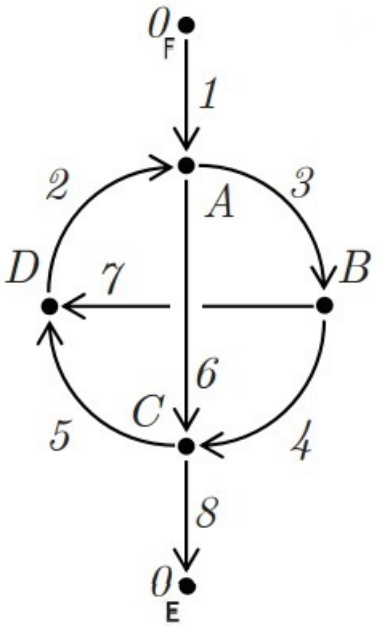
The obliged elements from 0_F to 0_E are in this case just *1, A, C, 6*. The only metabolite that has more than one outgoing arrow is A, namely reactions 2 and 5, both in the $A0$-direction. The first obliged element from A to 0 is C. Hence reactions 2 and 5 share the same set of influence (cfr: Chapter 7), which is the undirected cycle between the A and C, namely reactions {2,3,4,5}. The other reactions in this cycle, 3 and 4, do not influence anything since they are the only outgoing reaction from their mother metabolite.

Example III



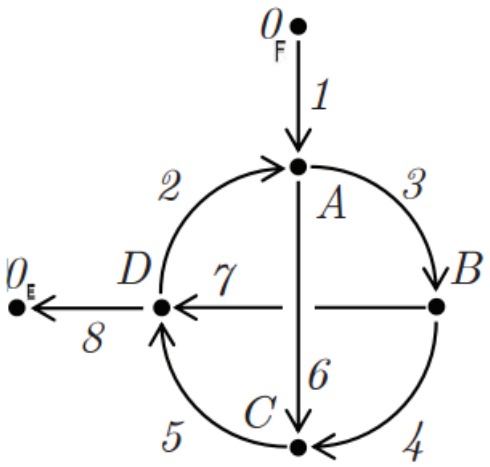
In this case we want to focus on metabolite D. It has exactly two outgoing arrows: reaction 5 in the $D0$-direction and reaction 8 not in the $D0$-direction. Reaction 5 is then also the first obliged element from metabolite D to 0. We know that reactions 5 and 8 do influence the same set, which in this case is the set $R^{D,8}$ of reactions reachable from D with a directed path starting along reaction 8, namely reactions: {8,9,3,6,7,2}. These two reactions, 5 and 8, are then of course also the peak of the influence pyramid, the 'absolute influencer', if we exclude from this analysis the exceptional case of the feed reaction 1. Note that the fact that reactions 5 and 8 share the same influenced set does not imply that they do influence each other in an equal way. Indeed in this case reaction 5 influences 8, but reaction 8 does not influence reaction 5. Note also that the $(D, 0)$-Balloon B_0^D in this case coincides precisely with $R^{D,8}$.

Example IV



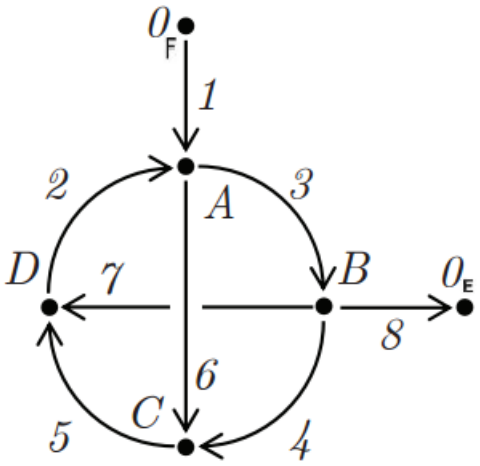
Here again we can apply an identical argument as above on metabolite C and its two outgoing reactions 5 and 8, which are the peak of the influence-pyramid. Reaction 2 is the only outgoing arrow from metabolite D and therefore does not influence anything and it is at the bottom of the pyramid. Apart from this, the first shared obliged element for the remaining metabolites, A and B, is C. Therefore reactions 5 and 8, outgoing from C, cannot be influenced by outgoing reactions of A and B. A has two outgoing reactions: 6 and 3, both in the $A0$-direction and B has also two outgoing reactions: 4 and 7, both also in the $B0$-direction. The $(A, 0)$-Balloon B_0^A coincides with the $(B, 0)$-Ballon B_0^B and contains reaction $\{2, 3, 4, 6, 7\}$. Note, however, that the reaction of this shared Balloon in the $A0$-direction are reactions {6,3,4}. The one in the $B0$-direction are instead reactions {6,4,7,2}.

Example V



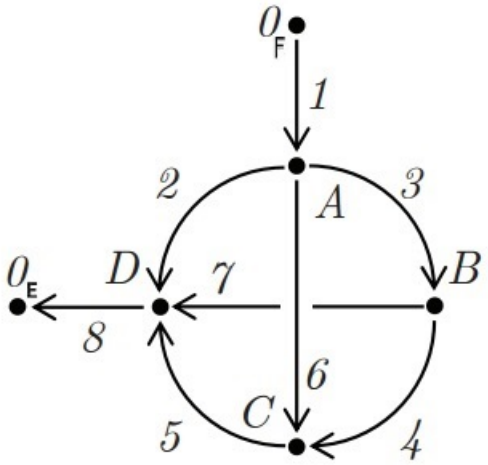
Again with the same arguments as the two examples before: the two outgoing reactions 8 and 2 from metabolite D are the peak of the influence-pyramid. Metabolite C has only one outgoing reaction 5 which is therefore at the bottom of the pyramid, namely it does not influence anything. Metabolites A and B share the same first obliged element, the metabolite D, and they both have two outgoing reactions in the 0-direction. The difference here with the previous example is that from B is not possible to reach A withouth passing through D namely withouth passing through an obliged element from B to 0. Therefore reactions 4 and 7, outgoing from B, do not influence reactions 6 and 3, outgoing from A. Indeed the reactions in the $(B, 0)$-Balloon are {4,5,7}. The reactions, instead, in the $(A, 0)$-Balloon are {3,4,5,6,7}.

Example VI



In this very interesting example we want firstly to focus on metabolite B. It has three outgoing reactions: 4, 7 and 8. Reaction 8 is in the $B0$-direction; reactions 4 and 7 are not. The first obliged element from B to 0 is of course reaction 8 itself. The influenced set of reaction 4 is made by the reactions reachable from B with a directed path starting along 4, namely reactions {4,5,2,3,6}. The influenced set of reaction 7 is made by the reactions reachable from B with a directed path starting along 7, namely {7,2,3,6,5}. Note that the two influenced sets are not the same due to reactions 4 and 7 itself. In similar situations (metabolite m with two outgoing reactions not in the $m0$-direction) the behaviour is identical: the two outgoing reactions cannot share the set of influence, due, in particular, to themselves. On the other hand, the set influenced by reaction 8 are the reactions in the $(B, 0)$-Balloon B_0^B which is made by the union of the sets influenced by reaction 4 and 7, namely reactions: {2,3,4,5,6,7}. Metabolites C and D have only one outgoing reaction, respectively 5 and 2. Therefore reactions 5 and 2 do not influence anything. Metabolite A has two outgoing reactions: reaction 3 in the $A0$-direction and reaction 6 not in the $A0$-direction. We know that the influenced set of these two reactions is the same (cfr: Chapter 7). The first obliged element from A is reaction 3 itself. Therefore reaction 3 influences reaction 6 but the opposite is not true. Then the influenced set of reaction 3, which is the same as the influenced set of reaction 6, is made by the reactions in $R^{A,6}$, the set reachable from A with a directed path starting along reaction 6, namely reactions {6,5,2}.

Example VII



Metabolite A has three outgoing reactions: {2,3,6}. These reactions are all in the $A0$-direction. Therefore we know that they influence exactly the same set (cfr: Chapter 7). The first obliged element from A to 0 is D. In conclusion these three reactions {2,3,6} influence the reactions in the $(A, 0)$-Balloon, that is reactions {2,3,4,5,6,7}. Metabolites C and D have just one outgoing reaction, respectively 5 and 8, which therefore do not influence anything. Moreover, since D is an obliged element from all the other metabolites to 0, its outgoing reaction 8 is not influenced by anything, except for the special case of the feed reaction 1. Metabolite B has two outgoing arrows, 4 and 7, which are both in the $B0$-direction. Therefore they influence the same set, that is the reactions in the $(B, 0)$-Balloon. Namely the reactions reachable from B without passing through the first obliged element from B to 0, which is D. In conclusion, the set made by reactions {4,7,5}.

Chapter 9

Discussion and conclusions

Discussion

At this stage of work, several questions were raised and are still open.

It is clear, in the pyramidal structure of flux-influence, which are the reactions that do not influence anything. These are indeed the reactions that are the only outgoing reaction from their tail metabolite. These reactions occupy the bottom of our pyramidal structure. Nevertheless, it is not clear which are the reactions which, in a monomolecular network, influence the most. And it is not in general completely clear if there exists a method for decomposing the network into subnetworks, which do not relate to each other in terms of flux-influence. In other words, and putting feed reactions aside, is the flux-influence pyramidal graph connected?

The structure of the influenced sets, or at least of the Balloons, is quite clear. Nevertheless, the precise assigment of exit reactions to 0, in a network, affects balloons and flux-influence relations, alike. We are lacking, at the present, a concise description of this important effect. See, however, consequences 1 and 4 in Ch.7 for flux-influence statements which are not affected by the precise positioning of other exit reactions.

Our work applies, at this stage, just to the monomolecular case. The vector case, which is more challenging, still has to be studied.

Conclusions

In the present thesis, we have presented an alternative point of view to the Fiedler-Mochizuki Theorem on flux-influence in the case of monomolecular reaction networks. We have provided indeed a new formulation to this theorem, which has, in our opinion, the following advantages:

1. to prove the transitivity of the flux-influence relation is easier and conceptually more transparent.
2. to provide in a straightforward way a clear characterization of the whole set of reactions influenced by the perturbation of a single one.

We have indeed proved the transitivity and characterized the influenced set of single reactions. We have derived also an inclusion result of the influenced sets: namely if reaction i is in the set $I(j)$ of the reactions influenced by a perturbation of reaction j, then $I(i) \subseteq I(j)$. This is, of course, nothing but the general version of the transitivity result, seen from an influenced set point of view.

We have also tried to glue together our point of view with the one of Hiroshi Matano, which was presented at the 2015 Equadiff Conference in Lyon in the talk: '*Balloons in chemical reaction networks*'.

Finally we have gather together some straightforward consequences that are very useful for applications.

Appendix: glossary of some definitions

The followings definitions are not complete. They present only a quick source of knowledge for readers who may want to recall quickly some concepts. After the definition, there is mentioned the chapter where, in the thesis, these definition were properly given.

1. **Tail of an arrow in a directed graph**: the vertex from which the arrow emanates.
   Cfr: Chapter 1.

2. **Head of an arrow in a directed graph**: the vertex toward which the arrow points.
   Cfr: Chapter 1.

3. **Directed Walk** (*Diwalk*): an alternating sequence of vertices and arrows such that, for any arrow of the sequence, the preceding and the following vertex are, respectively, its tail and its head.
   Cfr: Chapter 1.

4. **Directed path** (*Dipath*): a walk where no vertices and no arrows are repeated.
   Cfr: Chapter 1.

5. **Monomolecular reaction network**: a strict directed graph in which there is a special vertex, the vertex 0, such that from any other vertex m, called metabolite, there exists a directed path from m to 0.
   Cfr: Chapter 2.

6. **Last-minute shortcut**: if γ_1 and γ_2 are directed paths, respectively from x to y and from y to z, then the last-minute shortcut $s(\gamma_1\gamma_2)$ is a directed path from x to z.
   Cfr: Chapter 3, Definition 3.1.

7. **xy-direction subgraph**: the subgraph of all the directed paths from x to y.
   Cfr: Chapter 3, Definition 3.3.

8. **Obliged element from a metabolite m to 0**: the intersection of all the dipaths from m to 0, namely the cut-elements of the $m0$-direction.
   Cfr: Chapter 3, Definition 3.4.

9. **m-after**: let m be a metabolite and p, q be elements reachable from m. We say that q is m-after p if p is an obliged element from m to q.
   Cfr: Chapter 3, Definition 3.5.

10. **(m,0)-Balloon**: the set reachable from the metabolite m with a directed path that does not include the first obliged element from m to 0. Alternatively, the set reachable from m not m-after the first obliged element from m to 0.
    Cfr: Chapter 6.

Bibliography

[1] A. Mochizuki and B. Fiedler, Sensitivity of chemical reaction networks: a structural approach. 1. Examples and the carbon metabolic network, J. Theor. Biol. 2015 Feb 21;367: 189−202

[2] B. Fiedler and A. Mochizuki, Sensitivity of chemical reaction networks: a structural approach. 2. Regular monomolecular systems, Math. Meth. Appl. Sci. (2015)

[3] M. Feinberg, Chemical reaction network structure and the stability of complex isothermal reactors - I. The deficiency zero and deficiency one theorems. Chemical Engineering Science, Vol. 42, No. 10: 2229-2268 (1987)

[4] M. Feinberg, The existence and uniqueness of steady states for a class of chemical reaction networks, Arch. Rational Mech. Anal. 132 (1995) 311−170

[5] H. Matano, Balloons in chemical reaction networks. Equadiff Conference 2015 talk, Lyon.

[6] F. Harary, Graph Theory, Addison-Wesley, reading, MA, 1969

[7] J. A. Bondy and U. S. R. Murty, Graph Theory with Applications, North Holland (1976)

[8] K. Menger, Zur allgemeinen Kurventheorie. Fund. Math. 10: 96115 (1927)

[9] E. Lawler, 4.5. Combinatorial Implications of Max-Flow Min-Cut Theorem, 4.6. Linear Programming Interpretation of Max-Flow Min-Cut Theorem. Combinatorial Optimization: Networks and Matroids. Dover. pp. 117−120 (2001)

[10] C. H. Papadimitriou and K. Steiglitz, 6.1 The Max-Flow, Min-Cut Theorem. Combinatorial Optimization: Algorithms and Complexity. Dover. pp. 120−128 (1998)

Selbstständigkeitserklärung

Hiermit bestätige ich, Nicola Vassena, dass ich die vorgelegte Masterarbeit mit dem Thema

Monomolecular reaction networks: a new proof of flux transitivity

selbstständig angefertigt und nur die erwähnten Quellen und Hilfen verwendet habe. Die Arbeit ist erstmalig und nur an der Freien Universität Berlin eingereicht worden.

Berlin, den April 26, 2016.